OpenScope Assembly Instructions

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1 Introduction

OpenScope is a low-cost open-source microscope developed by the 2015 Cambridge-JIC iGEM team and based on Dr Richard Bowman's inverted bright-field microscope, the PiScope [1].

OpenScope is a modular microscope: The instructions in this booklet detail how to put together the basic microscope and explain the steps to add any of its modular components: fluoresence, dark field or motorising the stage.

A detailed bill of materials used in creating the OpenScope can be found at http://2015.igem.org/Team:Cambridge-JIC/Make_Your_Own .

2 Assembling the Basic Setup

2.1 Materials

The most basic version of OpenScope is controlled manually and is only capable of bright-field imaging. All necessary components are listed in Tables 1 and 2 below.

Table 1: 3D printed components

Amount	Part
1	Chassis
1	Base
2	Side supports for Z axis
1	Cube holder
1	z axis in 2 parts (vertical + horizontal)
1	3-legged camera holder
2	slide clips
1	bright-field cube (for RPi lens)
1	dark-field tube in 3 parts
3	manual gears
3	screw holders
3	motor-clip covers

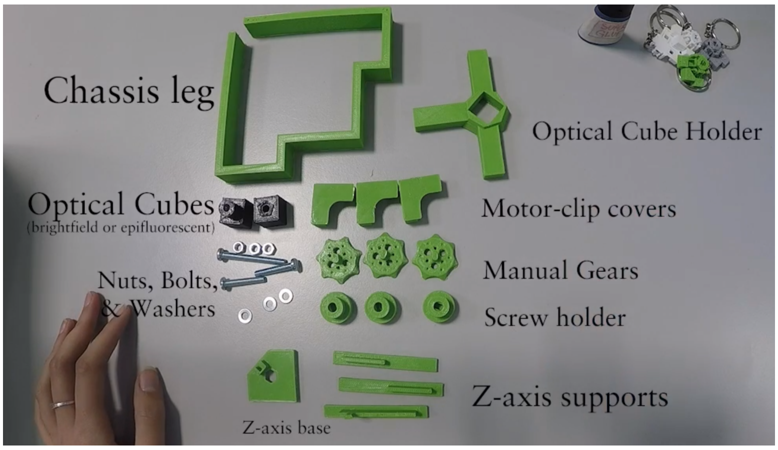
Table 2: Off-the-shelf components

Amount	Part
1	white LED (bright-field)
2	25 mm long M4 screws
1	40 mm long M4 screw
3	M4 nuts
3	M4 washers
1	Raspberry Pi 2 Model B
1	Ralink RT5370 WiFi adapter
1	Arduino UNO with its USB B connector
1	breadboard
1	16GB micro SD card
2	220 ohm resistors
1	ethernet cable
1	100 m spool insulated single-core wire
1	RPi camera
1	RPi ribbon cable
1	2A micro USB power cable

2.2 Instructions

1. Download the required files (http://2015.igem.org/Team:Cambridge-JIC/Downloads) and print them on a 3D printer. (See our 3D printing page for any troubleshooting - http://2015.igem.org/Team:Cambridge-

Figure 1: Parts for the OpenScope Chassis



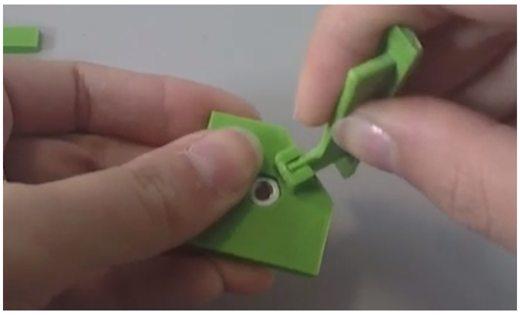
JIC/3D_Printing).

2. Turn the chassis upside down. Using superglue, glue two of the nuts to the base of the chassis (make sure to cut out the brim so they fit). Glue the third nut to the z-axis base; wait for the glue to dry and ensure the nuts are fixed in place.
3. Push the screws into the screw holders and glue them in. They need to be straight and fit tightly.

Figure 2: Glueing the nuts into the flexure system.

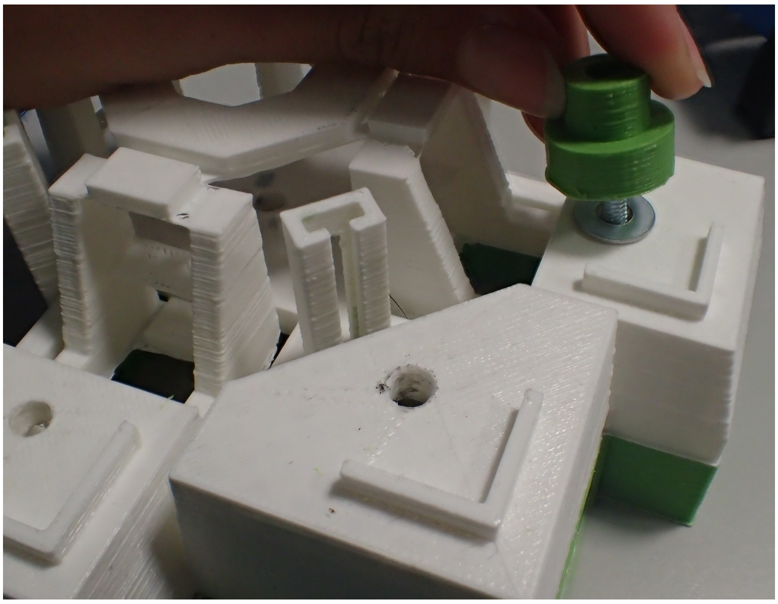
4. Glue the chassis leg(s) to the base.
5. Attach the z-axis to the z-axis base (there is a small slot) and glue the two together. make sure it stays straight.

Figure 3: Glueing the z-axis together.



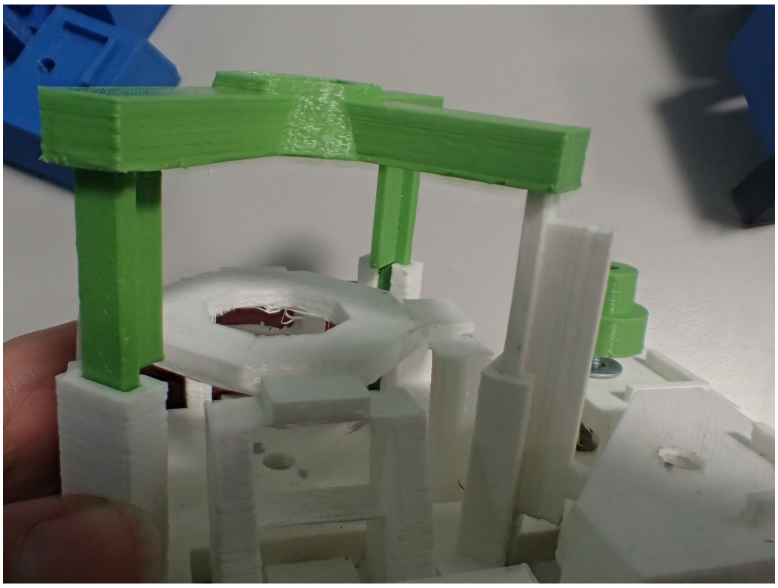
6. Place the washers on the screws, push two of the screws through the holes in the leg covers of the chassis and screw them into the nuts. (make sure they stay straight).

Figure 4: Sliding the screws into the chassis.



7. Slide the z axis into the chassis (from below) and screw the third screw in.
8. Click the optical cube holder into the side z axes, slide them in from above and click to the main z-axis.

Figure 5: Sliding in the z-axis. Note that the center axis is inserting from below.



9. Clip the handle covers to the stage - they are intended to prevent the screw tops from moving up. (Do not glue them in - you might have to take them off if you want to motorize the stage)
10. Click the manual gears into the holes on the top of the screw covers.
11. Remove the Raspberry Pi lens from it's CCD board (see our how to video at http://2015.igem.org/Team:Cambridge-JIC/Make_Your_Own) and slot the brightfield cube onto the CCD. The Raspberry Pi lens can be slotted into the brightfield cube. Place it with the large aperture facing outwards.
12. Set up the Arduino for with the white LED light. At this point a fluorescent LED is optional.
13. Finally, connect the arduino to the Raspberry Pi with the OpenScope image installed (see http://2015.igem.org /Team:Cambridge-JIC/Downloads#Software for the software).

Figure 6: Adding the manual gears onto the screw covers.

Figure 7: Fitting the brightfield cube onto the CCD.

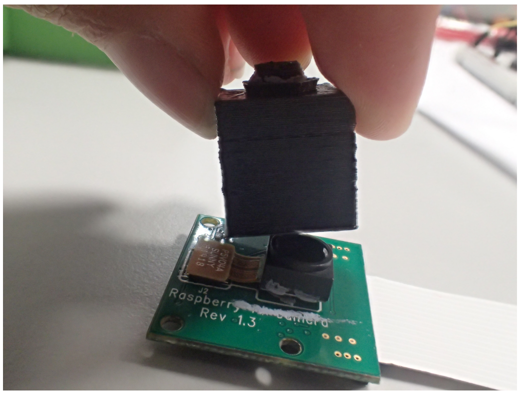
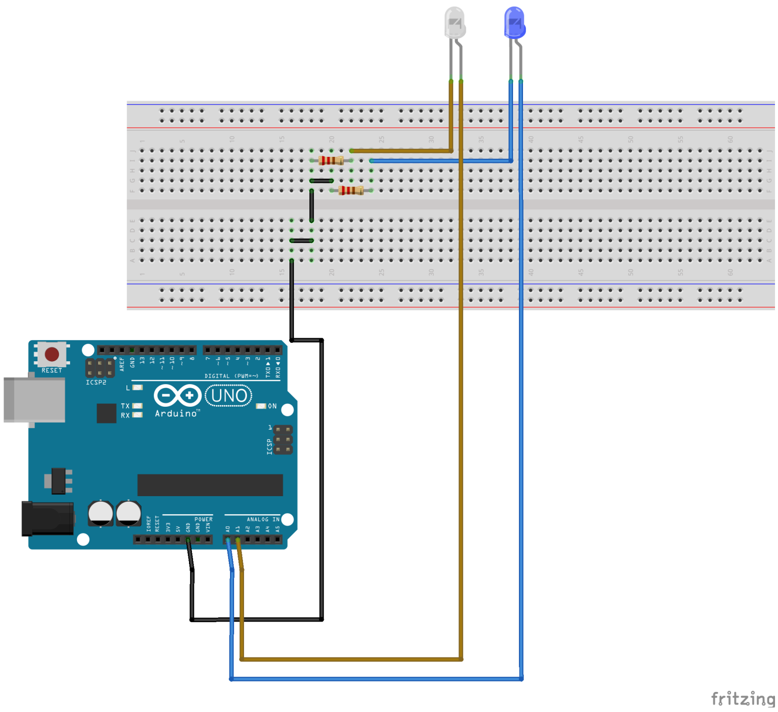


Figure 8: Wiring setup for manual microscope (no motors). This can be used for brightfield, darkfield and fluorescence.



3 Adding fluorescence

3.1 Materials

In order to image GFP fluorescence, the following items must be used. Note that different fluorescent proteins, will require different LEDs and filter sets.

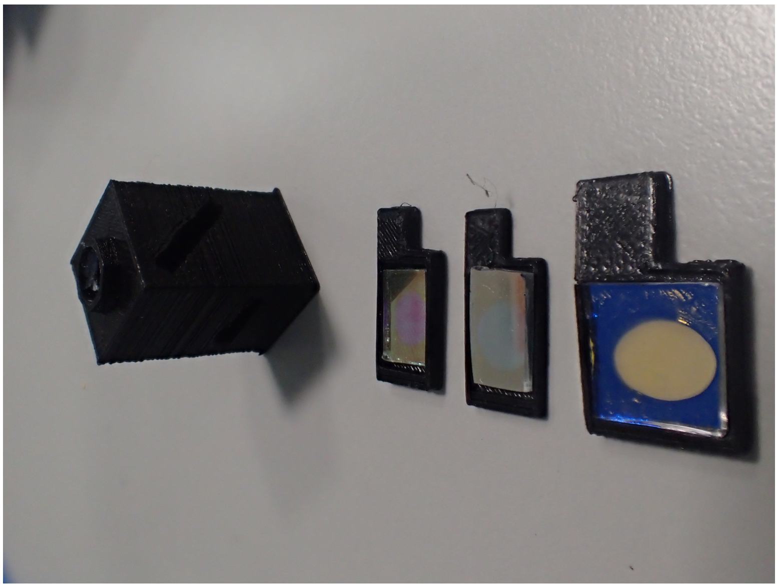
Table 3: 3D printed components for fluorescence

Amount	Part
1	Epicube (for Raspbery Pi lens)
1	Set of Drawers (for filters and mirrors)

Table 4: Off-the-shelf components for GFP fluorescence.

Amount	Part
1	Blue LED (GFP) Mfr: Kingbright; Part No. L-10934VBC/DS-D
1	550nm dichroic mirror, 25x16mm (GFP) Mfr: Comar; Part No. 550 1Y 116
1	490nm excitation filter, 25x16mm (GFP) Mfr: Comar; Part No. 495 1K 116
1	500nm emission filter, 25x16mm (GFP) Mfr: Comar; Part No. 515 1B 116

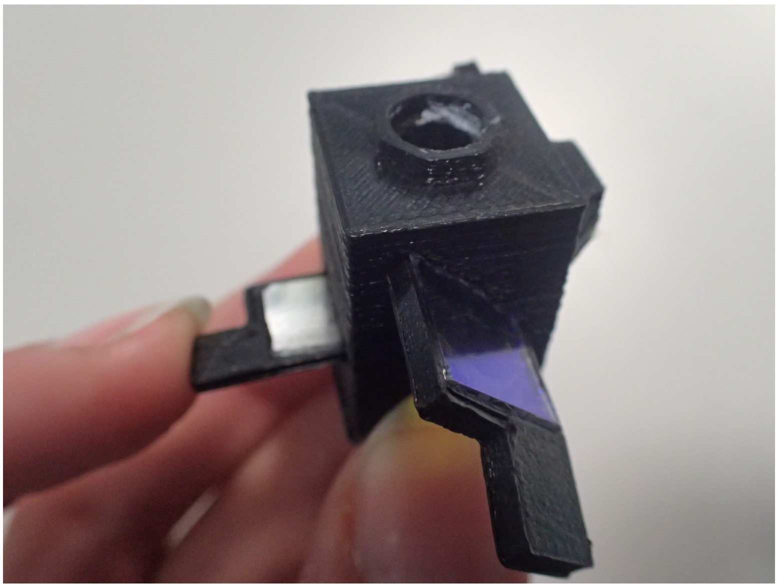
Figure 9: 3D components needed for epifluorescence.



3.2 Instructions

1. Print the cube and drawers for the filters that have been ordered. Be sure your filters are the right size for the cube or cut them yourself. The default sizes in our files are:
    - excitation filter: 11mm x 6mm
    - emission filter: 11mm x 7mm
    - dichroic mirror: 11mm x 10mm
2. Slot the filters drawers into the epicube and replace the brightfield cube with the epicube.
3. Include the Excitation LED in the Arduino setup (see Figure 8) and slot this LED into the epicube.

Figure 10: Filters being slotted into the epicube.



4 Adding darkfield

4.1 Materials

Table 5: 3D printed components for darkfield

Amount	Part
1	Darkfield tube (in 4 parts)

Table 6: Off-the-shelf components

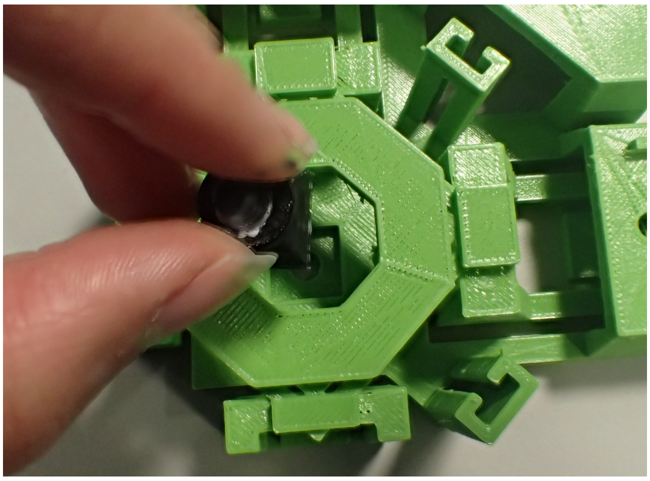
Amount	Part
1	Extra Raspberry Pi lens

4.2 Instructions

Darkfield imaging requires the central beam of illumination light to be blocked. This is achieved with the darkfield tube.

1. Print the darkfield tube: it comes out in 4 separate parts. Glue the two discs together: they serve to block the aperture. Connect the two cylindrical parts via these discs.
2. The bottom part of the darkfield tube clicks into a square hole in the base of the chassis (underneath the stage). It is easiest to do this from above, with the camera module unmounted. See Figure 11.
3. In the circular opening at the top of the tube place a Raspberry Pi camera lens with the small aperture facing outwards.
4. Also, invert the Raspberry Pi lens in the camera cube: place it with the small aperture facing outwards, too. This optimizes the lighting conditions.

Figure 11: Slotting the darkfield cube into the microscope.



5 Motorising the stage

5.1 Materials

For a motorised microscope, the following components must be added to the bill of materials:

Table 7: 3D printed components for motorisation

Amount	Part
3	screw holders
3	motor-clip holders

Table 8: Off-the-shelf components for motors

Amount	Part
3	28BYJ-48 Stepper motor
2	ULN2003A Darlington driver chips
1	9V battery connector

5.2 Instructions

1. Take out the manual gears and remove the screw covers. Replace them with the motor holders that click onto the stage.
2. Put the motors onto the holders (the rods of the holders go through the holes in the motors). For more stability, glue them on. Push the camshafts of the motors into the holes on the tops of the screw caps.
3. You will need to wire up the motors - see Figure 13. Test to see which motor corresponds to which axis on the Webshell.

Figure 12: Attatching the motor to the stage.

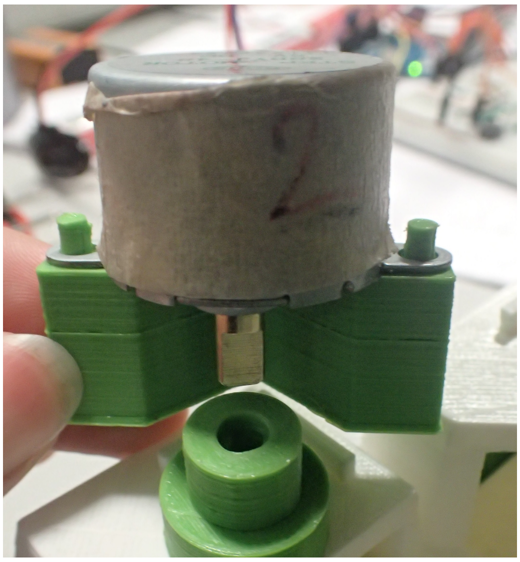
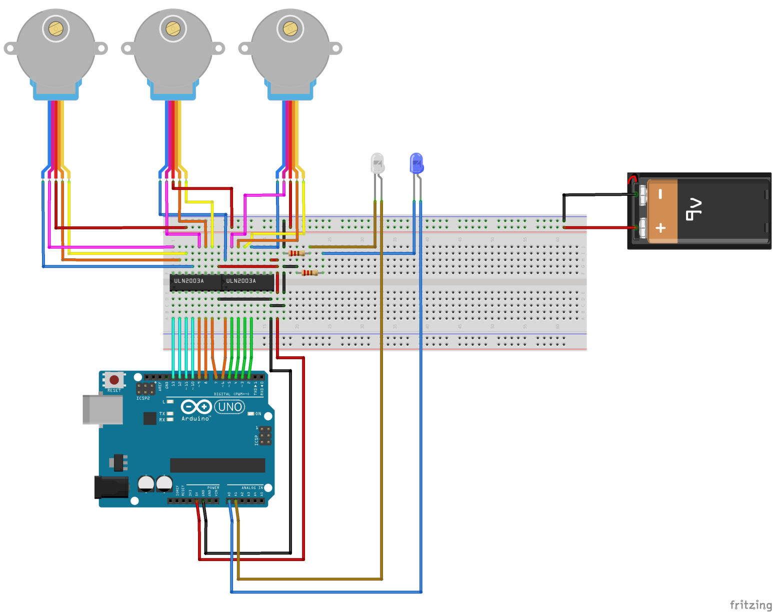


Figure 13: Wiring setup for motorising the stage.



6 Other additions

6.1 Changing the lens

Feel free to change the lens in your optics cubes. We have done a trial with a 4mm borosilicate ball lens to improve the resolution - to a submicron level. Note that sapphire lenses shouldn't be used due to their birefringent properties. This is compatable with fluorescence and brightfield, but has not been successful with darkfield.

- 1 Bright-field cube (for ball lens): 3D printed
- 1 Epicube (for ball lens): 3D printed
- 1 Borosilicate Ball lens: 12.20 GBP, Mfr: Comar; Part No: 03 VQ 04

6.2 Battery Powered

To make the microscope fully battery powered, add:

- 1 Extra battery holder for RPi: 3D printed

- 1 Extra 9V battery connector: 0.172 GBP; Brand: RS; Stock No. 489-021
- 1 Low Profile MoPi power board: 25.00 GBP; Product code: MOPI-LP

7 References

[1] James P. Sharkey, Darryl C. W. Foo, Alexandre Kabla, Jeremy J. Baumberg and Richard W. Bowman, *A one-piece 3D printed microscope and flexure translation stage*, online at: **arXiv:1509.05394 [physics.ins-det]**. (Accessed 18 Sep. 2015).